1250313

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November 17, 2004

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APPLICATION NUMBER: *60/510,358*
FILING DATE: *October 10, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/33194*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

PTO/SB/16 (08-03)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. ER 443070336 US

22151 U.S.PTO
60/510358
101003

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Ge Ming	Lui	55 South Kukui Street Apt 2810 Honolulu, HI 96813

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Composition and Methods for Cell Culturing

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number: 38551

OR

☐ Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 9
☐ Drawing(s) *Number of Sheets* _____
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number _____
☐ Other (specify) _____

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☑ A check or money order is enclosed to cover the filing fees.
☐ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Ge Ming Lui, PhD

TELEPHONE 808 949 2208

Date October 10, 2003

REGISTRATION NO. _____ *(if appropriate)*

Docket Number: _____

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	October 10, 2003
First Named Inventor	Ge Ming Lui
Examiner Name	
Art Unit	
Attorney Docket No.	

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	Fee Paid
Total Claims	-20** =		X		=
Independent Claims	- 3** =		X		=
Multiple Dependent					=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY

(Complete *(if applicable)*)

Name (Print/Type)	Ge Ming Lui	Registration No. (Attorney/Agent)	38551	Telephone	808 949 2208
Signature				Date	Oct 10, 2003

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Inventor: Ge Ming Lui
Patent Owner: Cellular Bioengineering, Inc.
1946 Young Street Suite 480, Honolulu, HI 96826 Phone: 808-949-2208

Patent 5: Composition and Methods for Cell Culturing

BACKGROUND ON VARIOUS STEPS

Title: 2E - A Novel Surface for Cell Growth, Coating, Expansion, and Transplantation

Background

Brief description of what this patent describes:
This patent describes the method of coating a polymer surface with Diamond Like Carbon (DLC) to render it useful as carrier for cells derived from neural origin.
What problem(s) does this address?:
It is very difficult to induce neurons to attach and grow onto polymer in tissue culture without the coating of neurotropic substance on the surface of the polymer.
How is this different from what's been done by others?:
Attempts are made to grow neurons on bare polymer surface without much success.
What future applications might this have?:
The polymer, either in the form of thin sheets or small beads, can be coated with DLC and be used as cell carriers for transplantation of neurons.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

DLC coating onto polymer will render it useful for carrying cells derived from neural origin. In combination with embedding a cocktail of attachment proteins-growth factors inside the matrix of the polymer, it may sustain the growth of nerve cells for a short period of time, up to several days. The polymer, either as a bead or a sheet, can act as a carrier for the implantation of nerve origin cells.
The implantation of DLC surface was done using a filtered vacuum-arc system. The vacuum-arc is formed by a high current discharge between two electrodes in vacuum. This produced abundance of carbon plasma from the cathode material., which carried the arc current. A repetitively plused vacuum-arc-plasma source was used with a pluse length of 5 ms and a repetition rate of 1/second. Any macroparticles was removed by a 90 degree magnetic filter mounted on a curved "magnetic duct" which stops line of sight transmission of macroparticles while allowing the transmission of plasma by virtue of an axial magnetic field. The substrate was mounted on a grounded holder about 10 cm from the duct outlet. The plasma gun or duct were at or near ground potential (within about 20 V). The film deposition is an energy deposition, resulting in a high quality, hydrogen free, diamond like carbon, not amorphous carbon or graphite.
The IP position in this case is the carrier function of the combination product, which is different from both the May Griffith and the Lawrence Berkeley patent.

Alternative steps or materials to address potential problems or if certain materials are not available:
Biodegradable polymers, when available, can be a more appropriate carrier for cell transplantation in the eye and the brain.
Better methods or compositions if new materials or complementary methods become available:
To embed the polymer with adhesive molecules/growth factors may enhance the biopolymer's ability to support neuron attachment and growth.

Inventor: Ge Ming Lui
Patent Owner: Cellular Bioengineering, Inc.
1946 Young Street Suite 480, Honolulu, HI 96826 Phone: 808-949-2208

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

1. To create a carrier for neural cells attachment by coating biopolymers with DLC for transplantation.
2. To use biodegradable polymer and coating it with DLC for the purpose of carrying neural cells for transplantation.
3. To embed the polymer with adhesive molecules/growth factors and then coating it with DLC to enhance neural cells attachment and growth and use it for cell transplantation.

****

Title: 3A - System for Nerve Cell Function Monitoring

Background

Brief description of what this patent describes:

This patent describes the creation of a self contained semi-solid polymer/gel block which contains nutrients and survival factors to sustain the growth of neurons to couple to a CCD chip for the detection of action potential signals.

What problem(s) does this address?:

When the neurons are grown on the CCD chip immersed in a liquid medium environment, attempts to measure the action potential via two electrodes introduced into the fluid causes shorting of the chip. The semi-solid polymer block will aloow the measurement without shorting out the chip.

How is this different from what's been done by others?:

Currently neurons are cultured in liquid medium. Therefore the CCD chip on which the neurons are grown onto is immersed in liquid.

What future applications might this have?:

Self contained units which can house and sustain the growth of neurons can be coupled into bio-driven computer chips.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Chip or CCD itself and its ideal characteristics for detection function.

A water-proof chip is needed for the culture of neural cells on it for detection of signals generated from neurons undergoing stimulation.

Alternatively, a self contained polymer/gel mixture with nutrients and survival factors for neurons can be used for coating the chip surface. The neurons will be cultured in the polymer/gel matrix until they are well developed, then the whole layer will be attached to the chip. assuming that the neurons can survive a period of time (up to several days) within this device, the action can be measured without shorting out the chip (since it does not immerse in fluid). The device is capable of being used as detection tool for nerve gases and other functions.

Alternative steps or materials to address potential problems or if certain materials are not available:

In order to induce the dendrites of the neurons to make contact with the chip, the surface of the chip can be pre-coated with Nerve Growth (NGF) or DLC.

Better methods or compositions if new materials or complementary methods become available:

A method to supply nutrients and survival factors for the neurons in the polymer/gel unit (by injection, for example) can lengthen the operational period of the polymer/gel unit.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

1. To create a semi-solid, self-containing polymer/gel unit for the growth of neurons.
2. To couple the polymer/gel unit with neurons grown on it with a CCD chip for detection of action potential after a stimulus is applied.
3. To coat the surface of the CCD chip with neural tropic substances (NGF and DLC) to enhance the contact between the dendrites and the chip.

****

Title: 3B - Composition and Method for Growth of Neural Cells on Various Surfaces

Background

Brief description of what this patent describes:

This patent describes a method to create a polymer/gel matrix with adhesion proteins/growth factors incorporated into it for supporting the growth, replication and differentiation of neural cells.

What problem(s) does this address?:

The growth of neural cells with contemporary tissue culture methods is highly unsuccessful. This problem may be solve by using a three dimensional substrate to embed the neural cells instead of growing them on a flat surface.

How is this different from what's been done by others?:

Most of the current attempts to grow neural cells are in a flat surface.

What future applications might this have?:

The ability to grow neurons in tissue culture will make it possible to perform neural cell transplantation. The neural cell cultures can also be used in drug discovery and drug testing, and to test and elucidate the mechanism of infections of neural tissues by micro organisms and viruses.

Specific Methods and/or Compositions

Step-by-step preferred method and/or description of composition:

Use of polymer to grow retinal ganglionic cells for chip technology combined with:

1. "May polymer" (any of the polymers created by the research group of May Griffith PhD, MBA, University of Ottawa Eye Institute, Ontario Canada)

May Griffith's polymer was reported to induce dendrite outgrowth from the nerve cells surrounding the cornea. This polymer may provide a three dimensional environment for the neurons to extend their dendrites or even proliferate. May has not shown evidence of nerve cell replication on this polymer yet. To test the possibility, retinal ganglionic cells are readily available and this cell type can replicate at lease 3 to 4 generations on BCE-ECM. The polymer can be used to grow this cell types and see if they can induce proliferation as well as neuronal differentiation.

2. "May polymer" + growth/attachment cocktail.
May polymer by itself may not be sufficient to support the growth, replication and differentiation of neuronal cells. When it is transplanted into the cornea, it may induce the extension of the dendrites (a tropic effect) but the body of the nerve cells are still situated in the surrounding host tissue. To enable the neurons to inhibit the polymer, a growth factor/attachment factor cocktail can be incorporated into the polymer during synthesis. The growth factors suitable for this purpose are bFGF at 50 ug/ml conjugated to polycarbophyll or heparan sulfate and NGF at 50 ug/ml also conjugated to polycarbophyll or heparan sulfate. The attachment factors needed are laminin at 500 ug/ml and RGDS at 500 ug/ml. These components can be added to the collagen type IV (which made up a large part of the May polymer) prior to the polymerization step.

3. "ideal polymer"
If another polymer (one that can be defined better structurally than the May polymer) is available, then it can be tested for the ability for supporting the growth, differentiation and proliferation of cells from neuronal origin. The same approach will be used for its application with the chip technology, i.e. to develop a coat or carrier to house the nerve cells for a period of time long enough for detection of signals.

4. "ideal polymer" + growth/attachment cocktail.
This is the same approach as in adding the growth factors/attachment factors onto the May polymer.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)
1. To make a polymer /gel matrix with adhesive proteins/growth factors incorporated into it to provide a three dimensional environment for the growth, proliferation and differentiation of neural cells.

****

Title: 3C - Composition and Method for Combining Polymers and Diamond Like Coatings for Cell Growth

Background

Brief description of what this patent describes:
This patent describes the coating of biopolymers with Diamond Like Carbon (DLC) for the culture of neural cells.
What problem(s) does this address?:
The current tissue culture techniques does not allow for the growth of neural cells in vitro. This problem can be addressed by providing a three dimensional environment for neural cell growth. The use of DLC coating may enhance the attachment and proliferation of the neural cells in culture.
How is this different from what's been done by others?:
Attempts to culture neural cells at present are using regular tissue culture dishes or flasks without the benefit of neurotropic coating.

What future applications might this have?:
The DLC coated polymer can be molded into the forms of small beads or thin sheets. These devices can act as carriers for neural cells transplantation.

Specific Methods and/or Compositions
Step-by-step preferred method and/or description of composition:

Use of any of polymers above with DLC.
The polymer can provide a three dimensional matrix for nerve cells to grow, the DLC will help the nerve cells to attach and proliferate. Combining the two technologies together may further enhance the culturing of cells derived from neural origin. The idea is to make a sheet or tiny beads with either the May polymer or another polymer of choice. The polymer sheet/bead will then be coated with DLC (assuming that the polymer can withstand the coating process) and the neural cells will be grown on the coated polymer. The sheet/bead can serve as carriers for cell transplantation in a lot of occasions.

Better methods or compositions if new materials or complementary methods become available:
To incorporate neurotropic factors (such as NGF and bFGF) into the polymer may further enhance the attachment, growth and differentiation of the neural cells.

Inventive Contribution, Improvements
(List all the points of this idea you feel are novel, critical, and/or patentable.)

1. To coat a biopolymer with DLC for use as carrier for neural cell transplantation.
2. To coat biopolymers containing neurotropic factors (NGF and bFGF) with DLC for use as culture device or carrier of neural cells in transplantation.

****

Title: 3D -Composition and Method for Combining Polymers and Extra Cellular Matrixes for Cell Growth

Background
Brief description of what this patent describes:
This patent describes the coating of biopolymers with extracellular matrix generated from bovine corneal endothelial cells for the growth of neural and other cell types.
What problem(s) does this address?:
This invention addresses the difficulties of growing human neural cells by conventional tissue culture techniques in vitro.
How is this different from what's been done by others?:
Current approach to grow human neural cells involves using tissue culture dishes and flasks without extra coating.
What future applications might this have?:
The ability to induce the growth and replication of neural cells in culture may make possible the banking of such cells for transplantation purpose in treatment of neural injury and disorders.

Specific Methods and/or Compositions
Step-by-step preferred method and/or description of composition:

Use of any of polymers above with ECM.
In a sheet or block form, the polymer can be coated with BCE-ECM for the growth of some neuronal cells. In order to achieve this aim, the polymer will have to be non-swollen when it is submerged in the culture media. The procedure calls for growing of the BCE cells on the polymer sheet/block for 7-10 days, then use 20 mM NH4OH to remove the BCE cells, leaving the ECM coated on the surface of the polymer. The resultant product can be used for neural cells as well as epithelial cells culture.

Better methods or compositions if new materials or complementary methods become available:

Addition of neurotropic factors (such as NGF and bFGF) into the polymer during synthesis may enhance its ability to support the growth and proliferation of neural cells.

Inventive Contribution, Improvements

(List all the points of this idea you feel are novel, critical, and/or patentable.)

1. The coating of biopolymers with extracellular matrix derived from bovine corneal endothelial cells to support the growth and differentiation of neural cells.
2. The coating of biopolymers containing neutropic factors (NGF and bFGF) with extracellular matrix derived from bovine corneal endothelial cells to support growth, proliferation and differentiation of neural cells.

****

THE LAW OFFICE OF JOSEPH G. CONTRERA, ESQ.
PATENTS, COPYRIGHTS, TRADEMARKS AND LICENSING

15004 BITTERROOT WAY ROCKVILLE, MD 20853-1711
PHONE: 240-988-8763 FAX: 301-929-1254 EMAIL: JOSEPH_CONTRERA@COMCAST.NET

October 6, 2003

Dr. Hank Wuh
Cellular Bioengineering, Inc.
1946 Young Street, Suite 480
Honolulu, HI 96826

Re: Draft Claims for Application 5
Compositions and Methods for Cell Culturing
Our Ref. No.: 1003-0005

This patent describes the method of coating a polymer surface with Diamond Like Carbon (DLC) to render it useful as carrier for cells derived from neural origin.

Claims for Application 5

Novel Compositions and Methods for Cell Culturing

1. An improved surface for the growth an attachment of cells comprising a biopolymer coated with a high quality, hydrogen free diamond-like carbon surface.

2. The improved surface of claim 1 wherein the biopolymer is biodegradable.

3. The improved surface of claim 1 wherein the biopolymer is in sheet form.

4. The improved surface of claim 1 wherein the biopolymer is in micro particle form.

5. A method of growing neurons in culture comprising the seeding and growth of neurons on a biopolymer coated with a high quality, hydrogen free diamond-like carbon surface.

6. The method of claim 5 wherein the biopolymer is biodegradable.

7. The method of claim 5 wherein the biopolymer is in sheet form.

8. The method of claim 5 wherein the biopolymer is in micro particle form.

9. The improved surface of claim 1 wherein the biopolymer has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate.

10. A method of growing neurons in culture comprising the seeding and growth of neurons on a biopolymer made using the method of claim 9.

11. An apparatus for detection of neural cell signals comprising:

a) a unit of biopolymer having embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with

polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes;

b) an integrated circuit chip or charge coupled device having a means for said neural processes or dendrites to make an electrical connection;

c) a detector means for measuring the electrical signals from the neurons; and

d) a means for attaching said chip to a detector means.

12. The apparatus of claim 11 wherein the biopolymer unit is self-contained.

13. The apparatus of claim 11 wherein the biopolymer unit is semi-solid.

14. The apparatus of claim 11 wherein the integrated circuit chip or charge coupled device has coated onto it during its synthesis, an attachment reagent comprising one or more of the following: Nerve Growth Factor or Diamond-Like-Carbon, to enhance the electrical contact between the neuronal processes or dendrites and the chip.

15. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth.

16. The growth medium of claim 15 further comprising "May Polymer".

17. The growth medium of claim 16 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.

18. The growth medium of claim 17 wherein the concentration of bFGF conjugated with polycarbophyll, or heparin sulfate is about 50 mcg/mL, the concentration of NGF conjugated with polycarbophyll, or heparin sulfate is about 50 mcg/mL, the concentration of laminin is about 500 mcg/mL and the concentration of RGDS is about 500 mcg/mL.

19. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth coated with Diamond-Like Carbon.

20. The growth medium of claim 19 further comprising "May Polymer".

21. The growth medium of claim 20 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve

Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.

22. The growth medium of any of claims 19-21 wherein said biopolymer is shaped into beads, sheets or micro-particles.

23. A method of transplanting neurons to a recipient host comprising the seeding of the neurons of interest into the growth medium of any or claims 19-21, allowing the neurons to grow to sufficient density, and implantation of the neurons within the growth medium into said host.

24. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth which is coated with BCE-ECM.

25. A method for making the growth medium of claim 24 comprising:

a) seeding onto said three dimensional growth medium at low density, a population of bovine corneal endothelial (BCE) cells in a culture media suitable for their growth;

b) allowing the BCE cells to grow to confluence; and

c) aspirating the media and treating the three dimensional growth medium with ammonium hydroxide for a sufficient period of time to remove the cells.

26. A three dimensional growth medium suitable for supporting the growth and replication of neural cells comprising a semi-solid biopolymer which is capable of supporting neuronal growth which is coated with BCE-ECM and with Diamond-Like Carbon.

27. The growth medium of claim 26 further comprising "May Polymer".

28. The growth medium of claim 27 wherein said "May Polymer" has embedded or incorporated into it during its synthesis, an attachment reagent comprising one or more of the following: laminin, fibronectin, RGDS, bFGF conjugated with polycarbophyll, EGF conjugated with polycarbophyll, and heparin sulfate or Nerve Growth Factor, sufficient to allow neural or nerve cells transplanted into said unit at low density to proliferate and send out neural processes.

28. The growth medium of any of claims 26-28 wherein said biopolymer is shaped into beads, sheets or micro-particles.

PTO/SB/01 (08-03)
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☑ Declaration Submitted With Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	
First Named Inventor	Ge Ming Lui
COMPLETE IF KNOWN	
Application Number	
Filing Date	October 10, 2003
Art Unit	
Examiner Name	

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name.

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Composition and Methods for Cell Culturing

(Title of the Invention)

the specification of which

☑ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) ☐ as United States Application Number or PCT International Application Number ☐ and was amended on (MM/DD/YYYY) ☐ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? Yes	No
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

PTO/SB/01 (06-03)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION — Utility or Design Patent Application

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR:		☐ A petition has been filed for this unsigned inventor	
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☐ Additional inventors or a legal representative are being named on the _____ supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

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